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Research Article

THE POTENTIAL IMPACTS OF THE ANTI-EPILEPTIC DRUG (OXCARBAZEPINE) ON ALBINO RAT'S NEONATES DURING LACTATION

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ABSTRACT

Objective: This study was undertaken to evaluate the potential risks of the anti-epileptic drug (oxcarbazepine [OXC]) administration on neonates.

Methods: The nursing rats orally administered from 7th day of gestation until the 28th day of lactation with 108 mg/kg OXC (human equivalent dose) daily. The neonates at day 7, 14, 21, and 28 of lactation were sacrificed and the postnatal developmental signs and skeletal malformation and the histopathology of liver, kidney, and brain of the pups were examined.

Results: Our results showed that OXC induced a significant reduction in the neonatal weight and length, delayed, weak and incomplete ossification, wavy ribs and the neonatal liver revealed histopathological changes, pyknotic hepatocytes, cytoplasmic vacuolization, dilated sinusoid, and necrotic area. Kidney revealed alternation changes, enlargement of the glomerulus, renal tubules degeneration, and lymphatic infiltration. Brain (cerebral cortex and cerebellum) showed neurodegenerative changes, vacuolization of neuropil, congested and dilated blood vessel and dark stain neurons. Biochemical studies showed that OXC induced a reduction in the level glutathione reduced an important intracellular antioxidant, and catalase (enzymatic antioxidant) compared to control group.

Conclusions: We support and proof the potential risks of the OXC administration on neonates.

Keywords: Rats, Lactation, Oxcarbazepine, Antiepileptic drug.

INTRODUCTION

Epilepsy is defined by the presence of recurrent, unprovoked seizures. A daily, long-term antiepileptic drug (AED) regimen is the typical treatment. People with epilepsy expect to participate fully in life experiences, including childbearing, as the majority of them have well-controlled seizures, and are otherwise healthy [1].

Breastfeeding is known for its beneficial effects on both mothers and infants [2,3]. Nevertheless, in mothers suffering from epilepsy or bipolar disorders treated with AEDs, some concerns on infant health may raise. The decision to encourage breastfeeding in those women should be taken after a careful evaluation of the possible side-effects on the infant caused by the indirect exposure to AEDs via breast milk [4].

Drugs usage during pregnancy and breastfeeding demand a critical evaluation regarding exposure period, dosage, therapy term, and fetal and neonatal susceptibility [5-8].

Oxcarbazepine (OXC) is a newer antiepileptic agent that has recently become increasingly used for childhood epilepsy [9].

OXC is a prodrug which is converted in its active metabolite (10,11-dihydro-10-hydroxy-carbazepine (monohydroxy derivative [MHD]), the clinically relevant metabolite of OXC in the liver. Reports of its use while breastfeeding are limited [4]. Due to insufficient information about the OXC drug, the aim of this study was to determine the potential risks of OXC in the neonates when nursing rats orally administered during the lactation period.

METHODS

Experimental animals

The present experimental study is thus carried out on the white albino rat (*Rattus norvegicus*). The standard guidelines of National Organization for Drug Control and Research were used in handling animals. Females of 11-13 weeks old, weighing 200-250 g were selected and vaginal smears were prepared every morning and examined under the light microscope according to the method [10] for 5 days to select the female with regular estrus. Two females with a regular estrus cycle were selected in the proestrus stage and caged together with one male (weighing 200-250 g) overnight under controlled environmental conditions of temperature, humidity, and light. The 1st day of gestation was determined by the presence of sperms in the vaginal smear [11].

Experimental procedure and dosing

Pregnant rats (n=20) were randomly divided into two groups (10 pregnant rats in treatment groups, 10 pregnant rats in the control group). The experimental groups were as follows: Group 1 (control group) received the ordinary drinking water was used as the control solution; Group 2 (OXC group) received 108 mg/kg/day of OXC (Trileptal [Novartis]). The dose of drug in the treatment Group 2 was defined according to the weights of the rats. Moreover, the drug dose was adjusted accordingly. The control solution and AED were administered orally, daily from 7th of gestation until 28th day of lactation via gastric tube. Water and food were supplied *ad libitum* during all the experiment.

Developmental observations

At the 7th, 14th, 21st and 28th day of lactation the neonates of two groups (A and B) were sacrificed. Neonatal body weight, body length, tail length, and external malformation were recorded.

Skeletal examination

Neonates were preserved in 95% ethyl alcohol and were stained with double staining of fetal skeletons for cartilage (alcian blue) and bone (alizarin red) according to the method described by Peters [12].

Histopathological preparation
On the 7th, 14th, 21st and 28th day of lactation respectively the neonates of the two groups (A and B) were sacrified by decapitation. Liver, kidney, and brain of neonates of different groups were fixed for histological examination by light microscopy in 10% formol saline for at least 24 hrs and then preserved in 70% ethyl alcohol. The neonates were dehydrated in ascending grades of ethyl alcohol, cleared in terpineol, and embedded in paraffin wax. Serial transverse sections 5 µ thick of different neonatal tissues were cut, mounted and stained with hematoxylin and eosin for general histological studies.

Oxidative stress investigation
Autopsy samples were taken from the brain of neonates in different groups were stored at −40°C for oxidative stress investigation. A piece of liver was weighted and homogenized in 10 mmol/L phosphate buffer saline as 10% (w/v) at pH 7.4. The homogenates were centrifuged and the supernatants were taken for the estimation of Glutathione reduced (GSH) and catalase (CAT).

Estimation of GSH reduced
Tissue GSH was determined by calorimetric method using reagent kits obtained from Biodiagnostic (Egypt) by the method [13].

Estimation of CAT
Tissue CAT was determined by calorimetric method using reagent kits obtained from Biodiagnostic (Egypt) by the method [14].

Statistical analysis
All the values were presented as means (µ) ± standard errors of the means comparison between more than two different groups was carried out using the one-way analysis of variance (ANOVA).

RESULTS

Effects of OXC on albino rat's neonates during lactation

External morphological studies

Growth retardation
The morphological examination of the neonates maternally treated with 108 mg/kg OXC showed growth retardation represented by a decrease in neonatal body weight, body length, and tail length compared with the control group on 7th, 14th, 21st, 28th day of lactation (Figs. 1-3).

Skeletal examination

Control group
The skeleton system (axial and appendicular) of control pups at 21st postpartum depression showed complete ossification of the bones comprises skull. The median dorsal series consists of paired bones; nasal (Na) roofing the nasal cavity, frontals (Fr), parietals (P), and single median bone (IP). Furthermore, upper jaw bones, premaxilla (Pm), and maxilla (Mx) were well ossified (Figs. 4a, 7a and 9a).

The vertebral column consists of several types of vertebra which are the main structure unit and each one composed of a centrum and a pair of lateral neural arches. There are five types of vertebrae with well-ossified centrums and neural arches, cervical (7), thoracic (13), lumbar (6), sacral (4) and caudal (28-30) according to the tail length (Figs. 5a and 8a).

The sternum composed of 6 well-ossified sternbrae. The last one called xiphisternum and connected to small cartilage named xiphoid cartilage (Fig. 11a).

The control pups have 13 pairs of thoracic ribs (ThR). Each one consists of well-ossified vertebral part and cartilaginous sternal part (Figs. 9b, 10b and 11b). The sternal portion of the ribs articulated with the sternum, while the last four pairs were freely, has not connection with the sternum, and named the floating ribs (Fig. 10a).

The pectoral girdle of the control neonates consists of well-ossified scapula (Sc) and clavical (C) on the ventral side and suprascapula on the

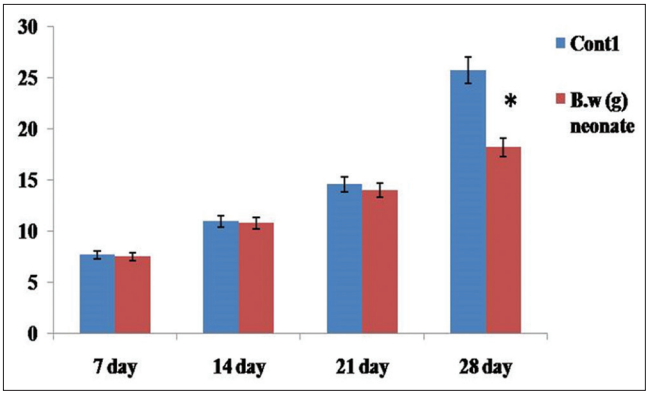

Fig. 1: A histogram showing effect of oxcarbazepine on the change in body weight (B.W) (g) of neonates maternally treated with 108 mg/kg from 7th of gestation to 7th, 14th, 21st, 28th day of lactation. Data are expressed as mean±standard error of mean (N=6). The statistical differences were analyzed by ANOVA followed by independent samples t-test. *Significantly different from control at p<0.05

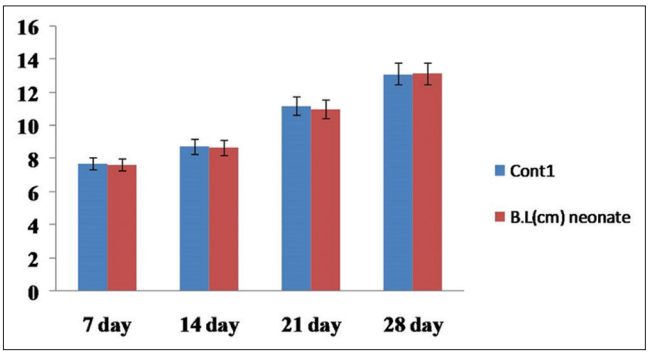

Fig. 2: A histogram showing effect of oxcarbazepine (OXC) on the change in body length (B.L) (cm) of neonates maternally treated with 108 mg/kg OXC on 7th, 14th, 21st, 28th day of lactation

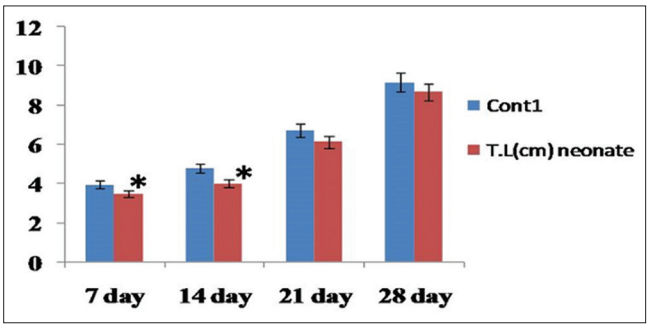

Fig. 3: A histogram showing effect of oxcarbazepine on the change in tail length (T.L) (cm) of neonates maternally treated with 108 mg/kg from 7th of gestation to 7th, 14th, 21st, 28th day of lactation

dorsal side which stained blue because it still cartilaginous (Fig. 8). The forelimb composed of well-ossified bones, humerus (Hu), radius (R), ulna (Ul), carpals (Ca), metacarpals (MC), and phalanges of five digits 1:2:2:2:2 (Figs. 5a and 8a).

The pelvic girdle of untreated pups consists of well ossified ilium (I), ischium (IS) and pubis (Op). The hind limb composed of well-ossified bones, femur (Fe), tibia (Ti), fibula (Fi), tarsals (Ta), metatarsals (MT), and phalanges of five toes 1:2:2:2:2 (Figs. 6a and 12a).

Treated group
The administration of a therapeutic dose of 108 mg/kg OXC induced some skeletal anomalies in both axial and appendicular skeleton

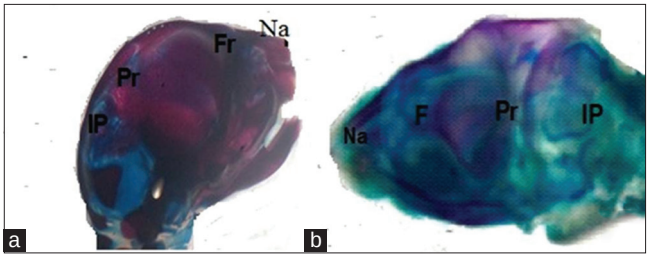

Fig. 4: (a) A photograph of skull of neonates of control pregnant rat at 7th day of lactation showing well ossification of nasal, frontal, parietal inter-parietal, squamosal and occipital bones, (b) a photograph of skull of neonates maternally treated with oxcarbazepine from 7th day of gestation to 7th day of lactation showing lack of ossification for nasal, frontal, parietals, inter-parietal

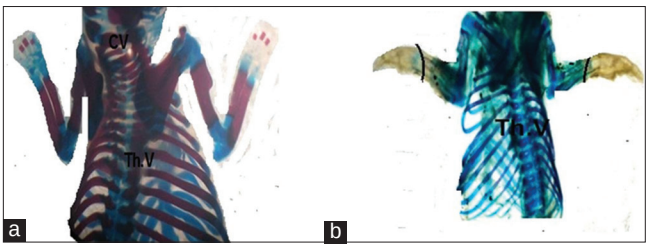

Fig. 5: (a) A photograph of skeleton of control neonates on 7th day of lactation showing normal ossification, (b) a photograph of skeleton of neonates maternally treated with oxcarbazepine from 7th day of gestation to 7th day of lactation showing the vertebral ribs of thoracic vertebrae sternabra, metacarpals and phalanges of the toes were completely unossified

compared to the control group that having skeleton with normal size and shape, all the bones in a well-ossified condition stained red. The neonates of the treated dams showed skeletal anomalies in the sternum, ribs, fore- and hind-limbs.

Neonates maternally treated with OXC from 7th day of gestation to 7th day of lactation. The skeletal abnormalities include delayed skeletal development shown as delayed ossification of skull bones (Fig. 4b), sternabrae. Thoracic vertebrae were either unossified or showed very small ossification centers and also its vertebral ribs. Moreover, no evident ossification centers for metacarpals, carpels bones and distal phalanges of fingers were found (Fig. 5b). Non-ossified centers of lumbar and sacral vertebrae. Moreover, the caudal vertebrae were absolutely non-ossified; bones of the pelvic girdle were mild ossified, no ossification centers for calcaneous, metatarsus or phalanges of toes (Fig. 6b).

Neonates maternally treated with OXC from 7th day of gestation to 14th day of lactation, mild ossification for parietals, inter-parietal, supraoccipital bones of skull (Fig. 7b). The metacarpals and phalanges of the fingers were completely weak ossified. Ribs the 12th and 13th ThRs were wavy ribs, the radius (R), and ulna (Ul) bones (Fig. 8b) were mild ossified the bones of lumbar, sacral, and caudal vertebra, bones of the pelvic girdle, tibia, fibula the metatarsus and phalanges of the toes were slightly ossified (Fig. 8c).

Neonates maternally treated with OXC from 7th day of gestation to 21st day of lactation, weak ossification for parietals, inter-parietal, supraoccipital bones of skull, ribs of the 13th thoracic vertebra and the first three sternabrae, while the last three were unossified (Figs. 9, 10 and 11b).

Neonates maternally treated with OXC from 7th day of gestation to 28th day of lactation, weak ossification for bones of skull, ribs of the 13th thoracic vertebra, and tibia and fibula bones (Fig. 12b).

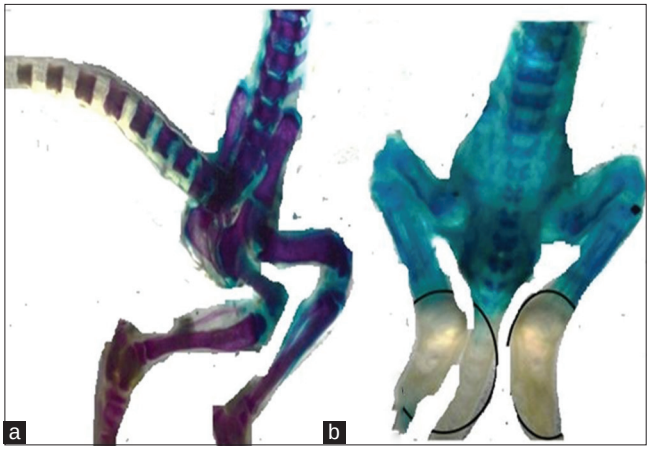

Fig. 6: (a) A photograph of skeleton of neonates of control pregnant rat showing complete ossification of lumber, sacral, caudal vertebrae and hind limb, (b) a photograph of skeleton of neonates maternally treated with oxcarbazepine from 7th day of gestation to 7th day of lactation showing non-ossified centers of lumbar and sacral vertebrae. Moreover, the caudal vertebrae were absolutely non-ossified; bones of the pelvic girdle were mild ossified

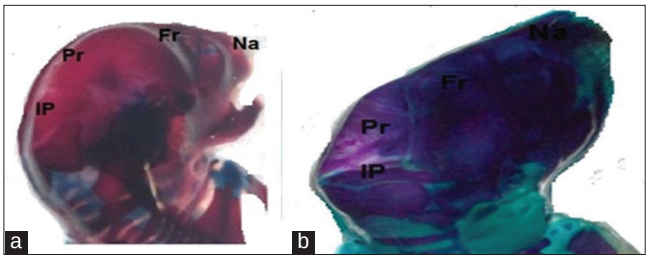

Fig. 7: (a) A photograph of skull of neonates of control pregnant rat at 14th day after delivery showing well ossified bones, (b) a photograph of skull of neonates maternally treated with oxcarbazepine from 7th day of gestation to 14th day of lactation showing weak ossified bones

Histopathological studies

Examination of serial transverse sections of the liver, kidney, and brain of albino neonates maternally treated with OXC showed some histopathological changes.

Liver of neonates

Control group

The liver of a control group showed a normal histological structure, where the liver appeared to compose of hexagonal or pentagonal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. The hepatocytes are arranged in trabecules running radiantly from the central vein and the spaces between the cell cords called blood sinusoids which converged toward the central vein and lined by Kupffer cells. Moreover, the hepatocytes are regular and contain a large spherical nucleus with a distinctly marked nucleolus and peripheral chromatin distribution. Some cells have two nuclei (Figs. 13a, c, e, g).

Treated group

The liver of neonates maternally treated with 108 mg/kg OXC from 7th day of gestation to 7th day of lactation showed degenerated hepatocytes, vaculation of the cytoplasm, lymphocytic infiltration, and congested vein filled with red blood cells (Fig. 13b). Congested portal vein with detached epithelial cell, portal vein wall invaded with lymphocytes. The liver of neonates at 14th day of lactation was showed degenerative and

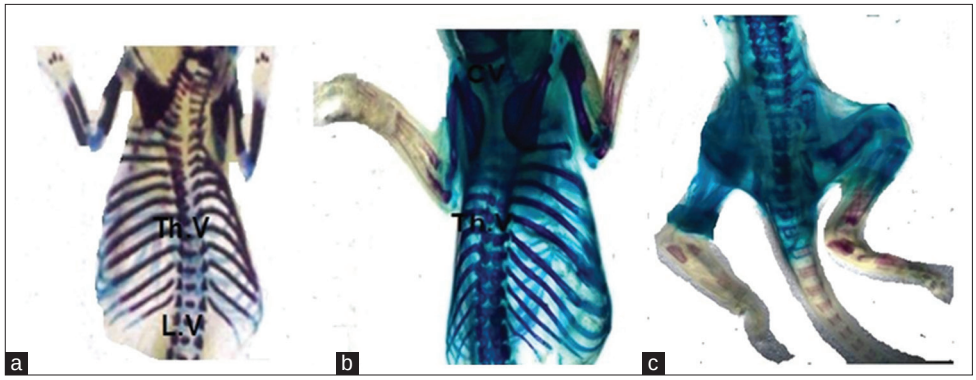

Fig. 8: (a) Photograph of skeleton of neonates of control pregnant rat at 14th day of lactation showing well ossification of fore limb, cervical, thoracic vertebrae and lumber vertebra, (b) a photograph of skeleton of neonates maternally treated with oxcarbazepine (OXC) from 7th day of gestation to 14th day of lactation showing metacarpals and phalanges of the toes were completely weak ossified. Ribs of were entirely non-ossified and were still in a cartilaginous state and the 12st and 13th thoracic ribs are wavy ribs, (c) a photograph of skeleton of neonates maternally treated with OXC from 7th day of gestation to 14th day of lactation showing The radius (R), ulna (Ul) bones. were mild ossified the bones of lumbar, sacral, and caudal vertebra, bones of the pelvic girdle, tibia, fibula the metatarsus and phalanges of the toes were slightly ossified

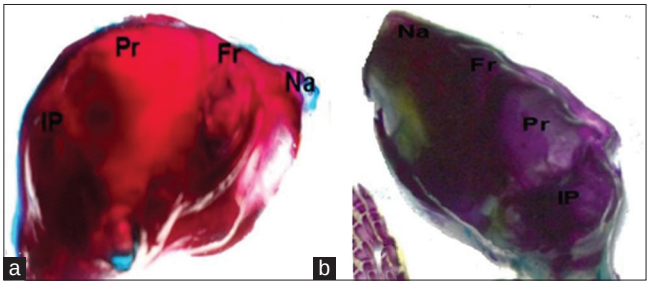

Fig. 9: (a) A photograph of skull of neonates of control pregnant rat at 21st day of lactation showing well ossified bones, (b) a photograph of skull of neonates maternally treated with oxcarbazepine from 7th day of gestation to 21st day of lactation showing weak ossified bones

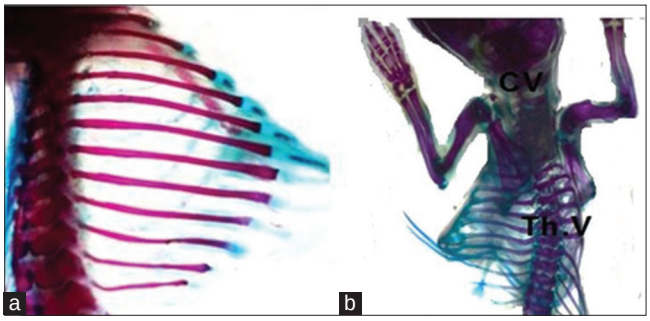

Fig. 10: (a) A photograph of skeleton of control neonates at 21st day of lactation showing complete ossification of ribs of thoracic vertebrae, (b) a photograph of skeleton of neonates maternally treated with oxcarbazepine from 7th day of gestation to 21st day of lactation showing weak ossification of ribs of 13th thoracic vertebra

necrosis of the hepatocyte. The cell membranes of most hepatic cells were disrupted and the cytoplasm of the other cells lost its normal characteristics (Fig. 13d). The liver of neonates at the 21st day of lactation showed severe hydropic, vaculation of the cytoplasm and some pyknotic nuclei (Fig. 13f). The liver of neonates at 28th day of lactation was showed congested portal vein that lined with thick epithelial cell wall. The cytoplasm of some hepatocyte showed rare empty vacuole-type spaces (Fig. 13h).

Kidney of neonates

Control group

Examination of kidney tissue of neonates from control nursing mother showed the normal structure of tissue. It was composed of two regions: An outer cortex and an inner medulla. The cortex consists of the Malpighian corpuscles and both proximal and distal convoluted tubules, while the medullary consists mainly of the descending and ascending limbs of Henle's loops. The collecting tubules are located in both the cortical and medullary regions. Each renal corpuscle is roughly spherical in shape and consists of the Bowman's capsule (BC) enclosing the glomerulus, a tuft of blood capillaries, and the proximal convoluted tubules are lined with typical thick cubic epithelium and the distal convoluted tubules show considerably lower cubic epithelium, the tubules have a relatively regular distinct lumen (Figs. 14a, c, e and g).

Treated groups

Examination of the kidney of neonates maternally treated with 108 mg/kg at different period of lactation, revealed various histopathological changes.

The kidney of neonates at the 7th day of lactation showed detatched cell of tubules, fatty degeneration, shrinkage glomerulus, abnormally cellular, and relatively vascular structure leaving rather wide capsular spaces. Degeneration of the epithelial lining of the tubules (Fig. 14b). The kidney of neonates at 14th day of lactation showed degenerated glomeruli within the BC and degeneration of tubules. Shrinking of the glomerular within BC and widening of the capsular space (Fig. 14d). The kidney of neonates at 21st day of lactation showed that the cell membrane of the lining epithelial cells of the distal tubules were disrupted and their cytoplasm appeared vacuolated, hemorrhagic foci were encountered between the renal tubules (Fig. 14f). In other section, the kidney of neonates at 28th day of lactation showed. Deterioration of the nuclei of the lining cells of the tubules and the lining cells of both tubules exhibit different stages of necrosis and pyknotized. The epithelial lining of the tubules exhibited cloudy swelling (Fig. 14h).

Brain of neonates

Control group

The brain tissue of neonates of control nursing rats showed normal architecture of all regions of brain sections (cerebral cortex, hippocampus, and cerebellum). Furthermore, revealed classic appearance of the pyramidal cell layer, granular cell layer, and Purkinje cell (Fig. 15a, c, e and g).

Treated group

Histological changes in varying degrees were observed in the brain of the neonates of the treated groups.

Section of cerebral hemispheres of neonates at 7th day of lactation showed dilated and congested vascular vessel and fibers accumulation. Degenerated area of cerebral cortex showed perivesicular neuron, vacuolization of neuropil and fibrosis. Many intensely stained "dark" and degenerated neurons are present in the cerebral cortex. Neuronal degeneration characterized by a heterogeneous pattern including shrunken eosinophilic neurons with pyknotic nuclei, vacuolated and spongiform neuropil (Fig. 15b). The brain of neonates at 14th day of lactation showed dark stained neuron, fibrosis, vacuolization, decreased in neurons numbers and spongiform neuropil. Degenerated neuron in the granular layer (GL) (Fig. 15d). The brain of neonates at 21st day of lactation showed cerebral cortex layers, molecular layer, GL with dark stained neurons, pyramidal layer with perivesicular neurons, and dilated blood vessels (Fig. 15f). The brain of neonates at 28th day of lactation showed dilatation of the vascular vessels. Degenerated, shrunken and dark neuron, thrombosis. Severe congested vascular vessel (Fig. 15h).

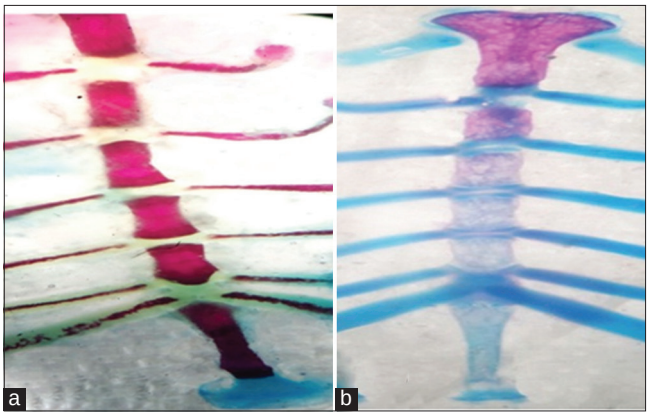

Fig. 11: (a) A photograph of control sternum of neonates at 7th day after delivery showing complete ossification of sternbrae bones, (b) a photograph of sternum of neonates maternally treated with oxcarbazepine from 7th day of gestation to 21st day of lactation showing weak ossification of the first three sternabrae, while the last three were unossified

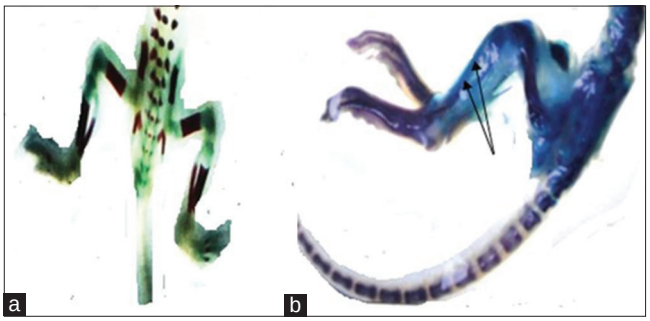

Fig. 12: (a) A photograph of skeleton of neonates of control pregnant rat showing complete ossification of lumber, sacral, caudal vertebrae and hind limb, (b) a photograph of skeleton of neonates maternally treated with oxcarbazepine from 7th day of gestation to 28th day of lactation showing weak ossification of tibia and fibula (arrow)

Oxidative stress observations

The effect of oral administration of OXC during gestation until different periods of lactation on the level of some non-enzymatic antioxidant defense system as GSH, enzymatic antioxidant defense system such as CAT in their neonates.

Neonates maternally treated with 108 mg/kg from 7th of gestation to 7th, 14th, 21st, 28th day of lactation indicated a marked decrease in brain GSH and CAT content throughout the experiment compared to control neonates (Table 1).

DISCUSSION

As with most other AEDs [15], OXC and its active metabolite MHD are excreted into breast milk. Although a small number of case reports showing no effects of OXC exposure via breast milk on infant development were identified [16-18].

The present study was carried out to evaluate the potential risks of the OXC on the neonates of albino rats orally administrated with 108 mg/kg during the lactation period.

Treatment with 108 mg/kg (HED) OXC caused reduction in body weight, body length and tail length of neonates when compared with the control. The observed growth retardation may be due to direct action of the used OXC drug on neonates and their tissues, or may be the drugs monitor them for decreased feeding.

These findings are in agreement with the study [19] that the drugs monitor the infant for drowsiness and decreased feeding, and developmental milestones especially in the first 2 months of life.

The neonates whose mothers treated with 108 mg/kg (HED) of OXC during lactation period showed no morphological anomalies appeared compared with those of control.

Neonates of maternally administrated with OXC showed decreased in the degree of ossification of the bones, the wavy thoracic ribs. Incomplete ossification of major bones may be due to alternation in calcium metabolism or reduction of calcitonin level in the developing offsprings so that the later causes lead to weak development in bones.

These findings are in agreement with Many studies have shown a significant reduction in bone mineral density in patients treated with new (OXC) AEDs. In spite of data about the possible effects of the AEDs on calcium metabolism, The abnormalities of calcium metabolism were thought to result from the cytochrome P450 enzyme-inducing properties of some AEDs and the resultant reduction in vitamin D levels [20,21].

Table 1: Effect of 108 mg/kg OXC on brain CAT (U/g) and GSH (mg/g) of neonates at 7th, 14th, 21st, 28th day of lactation

Groups during lactation	CAT (U/g)				GSH (mg/g)			
	7th day	14th day	21st day	28th day	7th day	14th day	21st day	28th day
Control group	0.506±0.014	0.685±0.015	0.550±0.008	0.625±0.012	0.038±0.008	0.042±0.007	0.054±0.009	0.044±0.003
Groups maternally treated with OXC	0.503±0.01	0.526±0.04*	0.5083±0.008*	0.558±0.007*	0.027±0.004	0.0381±0.009	0.050±0.006	0.041±0.003

Data are expressed as mean±SEM (n=6). The statistical differences were analyzed by ANOVA followed by independent samples t-test. *Significantly different from control at p<0.05. OXC: Oxcarbazepine

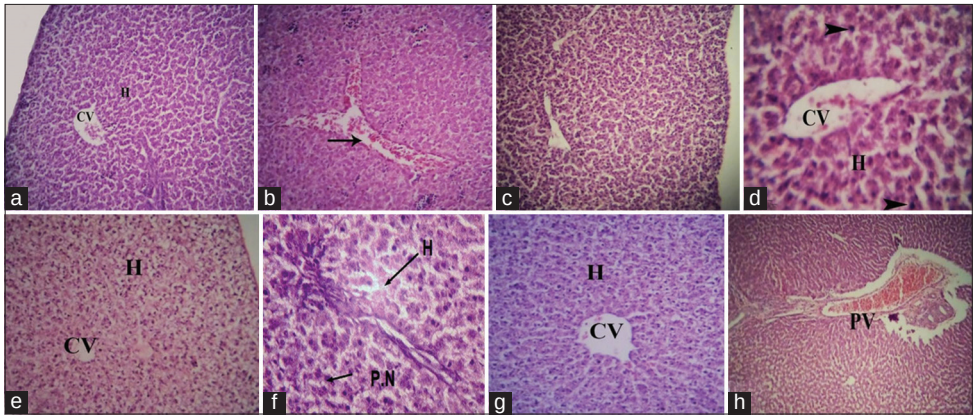

Fig. 13: (a) A photomicrograph of a section of liver of control neonates at 7th day of lactation showing no histopathological alteration, observed and the normal histological structure of the central vein (C.V.) and surrounding hepatocytes (H) were recorded. H and E stain, (b) a photomicrograph of a section of liver of neonates treated with 108 mg/kg oxcarbazepine (OXC) from 7th day of gestation to 7th day of lactation after delivered showing branched and congested vein filled with red blood cells (arrow). H and E stain, ×100, (c) a photomicrograph of a section of liver of a control neonate until 14th day of lactation showing no histopathological alteration observed and surrounding hepatocytes were recorded. H and E stain, ×100, (d) a photomicrograph of a section of liver of neonates treated with 108 mg/kg OXC at 14th day of lactation showing hepatocyte exhibit sever fatty degeneration, some hepatocyte are pyknotic (Head arrow). H and E stain, ×400, (e) a photomicrograph of section of liver of control neonates until 21st day of lactation showing normal architecture of the liver tissue. The central vein with its intact endothelial lining (CV). The hepatic lobules that can be only distinguished by their central veins, hepatocytes (G) and blood sinusoids. H and E stain, ×100, (f) a photomicrograph of section of liver of neonates treated with 108 mg/kg OXC at 21th day of lactation. Showing severe hydropic (H.D.), vaculation of the cytoplasm (V) and some pyknotic nuclei (P.N.). H and E stain, ×400, (g) a photomicrograph of section of liver of control neonates until 28th day after delivered showing normal architecture of the liver tissue. The central vein with its intact endothelial lining (CV). The hepatic lobules that can be only distinguished by their central veins, hepatocytes (H). H and E stain, ×100, (h) a photomicrograph of section of liver of neonates treated with 108 mg/kg OXC from 7th day of gestation to 28th day of lactation showing severe congested portal vein that lined with thick epithelial cell wall. H and E stain, ×100

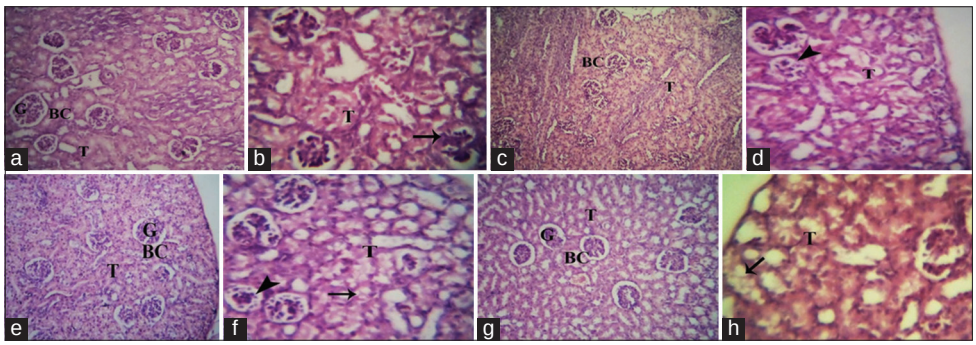

Fig. 14: (a) A photomicrograph of a section of kidney of neonates of 7th day of lactation showing the normal structure of renal tissue cortex and medulla, Bowman's capsule (BC), glomerulus (G), proximal and distal convoluted tubules (T) and collecting tubule. H and E stain, ×100, (b) a photomicrograph of a section of kidney of neonates treated with 108 mg/kg oxcarbazepine (OXC) from 7th to 20th day of gestation until 7th day of lactation showing congestion and shrinking of the glomerular (G), degeneration, cloudy swelling of the epithelial lining of the tubules (T). H and E stain, ×400, (c) a photomicrograph of a section of kidney of neonates of 14th day after delivered showing the normal structure of renal tissue cortex and medulla, BC, glomerulus (G), proximal and distal convoluted tubules (T) and collecting tubule. H and E stain, ×100, (d) a photomicrograph of a section of kidney of neonates treated with 108 mg/kg OXC from 7th day of gestation to 14th day of lactation showing degenerated glomeruli within the BC (head arrow) and degeneration of tubules (T). H and E stain, ×100, (e) a photomicrograph of a section of kidney of control neonates until 21th day after delivered showing the normal structure of renal tissue cortex and medullaes, Bowman's capsule (BC), glomerulus (G), proximal and distal convoluted tubules (T) and collecting tubule. H and E stain, ×100, (f) a photomicrograph of a section of kidney of neonates treated with 108 mg/kg of OXC from 7th day of gestation to 21st day of lactation showing, degeneration of the epithelial cell wall of tubules (T), degenerated glomeruli (head arrow) and cytoplasmic vacuoles (arrow). H and E stain, ×100, (g) a photomicrograph of section of kidney of control neonates until 28th day after delivered showing part of cortical region containing, a glomeruli (G) within (BC) and both proximal and distal tubules (T). H and E stain, ×100, (h) a photomicrograph of section of kidney of neonates treated with 108 mg/kg OXC from 7th day of gestation to 28th day of lactation. showing deterioration of the nuclei of the lining cells of the tubules and the lining cells of both tubules exhibit different stages of necrosis and pyknotized (T). H and E stain, ×400

The present study indicated that OXC induced marked histopathological changes in the liver, kidney, and brain. Musso *et al*. [22] hypothesized that OXC could induce hyponatremia as a consequence of its influence on distal nephron where it could promote free water retention, urinary sodium loss, or both of them. A greater tubular sensitivity to OXC influence.

These findings are in agreement with the study [23] that levetiracetam administration caused shrinking in glomeruli, acute cellular swelling in lining epithelium of the tubules at the cortex and hydropic degeneration in kidney and also increased histopathological changes in liver and brain tissues during lactation.

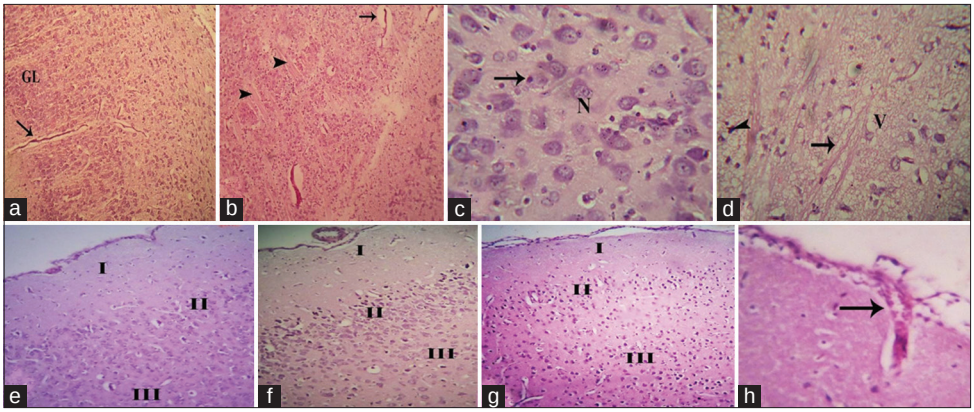

Fig. 15: (a) A photomicrograph of a section of control brain (cerebral hemisphere) of neonates of 7th day after delivered showing granular layer (GL), blood vessel (arrow) with normal width. H and E stain, ×100, (b) a photomicrograph of a section of brain (cerebral hemisphere) of neonates treated with 108 mg/kg oxcarbaziben from 7th to 20th day of gestation until 7th day after delivered showing dilated and congested vascular vessel (arrow) and fibers accumulation (head arrow), H and E stain, ×100, (c) a photomicrograph of a section of brain (cerebral hemisphere) of neonates of 14th day after delivered showing normal shape neuron (N) and microglia (arrow), H and E stain, ×400, (d) a photomicrograph of a section of brain (cerebral hemisphere) of neonates treated with 108 mg/kg oxcarbaziben from 7th to 20th day of gestation until 14th day after delivered showing dark stained neuron (head arrow), fibrosis (arrow), vacuolization (V), decreased in neurons numbers and spongiform nouropil. H and E stain, ×400, (e) a photomicrograph of a section of brain (cerebral hemisphere) of neonates of 21st day after delivered showing cerebral cortex layers. I=Molecular layer, II=Granular layer, III=Pyramidal layer. H and E stain, ×100, (f) a photomicrograph of a section of brain (cerebral hemisphere) of neonates treated with 108 mg/kg oxcarbaziben from 7th day of gestation to 21st day of lactation showing cerebral cortex layers. I=Molecular layer, II=Granular layer with dark stained neurons, III=Pyramidal layer with perivesicular neurons, ×100, (g) a photomicrograph of a section of brain of neonates of 28th day after delivered in control group showing cerebral cortex layers. I=Molecular layer, II=Granular layer, III=Pyramidal layer. H and E stain, ×100, (h) a photomicrograph of a section of brain (cerebral hemisphere) of neonates treated with 108 mg/kg oxcarbaziben from 7th day of gestation to 28th day of lactation showing severe congested vascular vessel (arrow), H and E stain, ×400

The present study indicated that OXC induced a marked decrease in brain GSH and CAT content throughout the experiment compared to control neonates.

AEDs in therapeutic dosage have been known to unfavorably alter the redox balance in experimental models and in human beings [24,25].

These data indicated that OXC induced oxidative stress in rat brain.

These findings are in agreement with the study [26] found that OXC significantly increased lipid peroxidation levels and significantly decreased levels of reduced GSH in rats with pentylenetetrazole-induced epilepsy.

Excess oxidative stress can be a final common pathway, by which AEDs exert teratogenic effects [27].

These data indicated, in our study, in disagreement [4] considered that OXC moderately safe in the breastfeeding mother.

In view of the data of the present study, it can deduce that OXC has been distributed in pup's tissues through the milk of lactating and caused oxidative damage, brain, liver, and kidneys dysfunction in neonates.

CONCLUSION

It can be concluded that the administration of OXC during lactation should only be considered if the expected benefit to the mother is greater than any possible risk to the neonate.

REFERENCES

1. Harden CL, Meador KJ, Pennell PB, Hauser WA, Gronseth GS, French JA, *et al*. Practice parameter update: Management issues for women with epilepsy – focus on pregnancy (an evidence-based review): Teratogenesis and perinatal outcomes: Report of the quality standards subcommittee and therapeutics and technology assessment subcommittee of the American Academy Of Neurology and American Epilepsy Society. Neurology 2009;73(2):133-41.
2. Hale TW. Medications and Mother's milk. 15th ed. 1712 N Forest Amarillo TX 79106, USA: A Manual of Lactational Pharmacology; 2012. p. 184-6, 264-5, 325-6, 426-7, 495-6, 651-3, 664-5, 870-1, 905-6, 910-1, 938-9, 940-1, 1077, 1091-2, 1126-7, 1136-7, 1161-2, 79106-7017.
3. Section on breastfeeding. Breastfeeding and the use of human milk. Pediatrics 2012;129(3):e827-41.
4. Davanzo R, Dal Bo S, Bua J, Copertino M, Zanelli E, Matarazzo L. Antiepileptic drugs and breastfeeding. Ital J Pediatr 2013;39:50.
5. Sá del Fiol F, Gerenutti M, Groppo FC. Antibiotics and pregnancy. Pharmazie 2005;60(7):483-93.
6. Iqbal MM, Sohhan T, Mahmud SZ. The effects of lithium, valproic acid, and carbamazepine during pregnancy and lactation. J Toxicol Clin Toxicol 2001;39(4):381-92.
7. Mantovani A, Calamandrei G. Delayed developmental effects following prenatal exposure to drugs. Curr Pharm Des 2001;7(9):859-80.
8. Gerenutti M, Spinosa HS, Bernard MM. Some considerations on the toxicology of development. Scientific Communications Faculty of Veterinary Medicine and Animal Science, University of SãoPaul 1991; 15(1):27-9.
9. Bang L, Goa K. Oxcarbazepine: A review of its use in children with epilepsy. Paediatr Drugs 2003;5:557-73.
10. Snell GD. Biology of the Laboratory Mouse, 5th ed. Pholadeliphia, PA: The Blakiston Company; 1956.
11. McClain RM, Becker BA. Teratogenicity, fetal toxicity, and placental transfer of lead nitrate in rats. Toxicol Appl Pharmacol 1975;31:72-82.
12. Peters PW. Double staining of fetal skeletons for cartilage and bone. In: Neubert D, Merker HJ, Kwasigroch TE, editor. Methods in Prenatal Toxicology. Stuttgart: Georg Thieme; 1977. p. 153-4.
13. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-8.
14. Aebi H. Catalase *in vitro*. Methods Enzymol 1984;105:121-6.
15. Bar-Oz B, Nulman I, Koren G, Ito S. Anticonvulsants and breast feeding: A critical review. Paediatr Drugs 2000;2(2):113-26.
16. Gentile S. Oxcarbazepine in pregnancy and lactation. Clin Drug Investig 2003;23(10):687.
17. Bülau P, Paar WD, von Unruh GE. Pharmacokinetics of oxcarbazepine and 10-hydroxy-carbazepine in the newborn child of an oxcarbazepine-treated mother. Eur J Clin Pharmacol 1988;34(3):311-3.

18. Pedersen B. Oxcarbazepine in Breast Milk. Abstract presented at the 17th. Jerusalem, Israel: Epilepsy International Congress; 1987. p. 6-11.
19. Drugs and Lactation Database (LactMed). United States National Library of Medicine; 2013. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?LACT. [Last accessed on 2013 Apr 07].
20. Babayigit A, Dirik E, Bober E, Cakmakci H. Adverse effects of antiepileptic drugs on bone mineral density. Pediatr Neurol 2006;35(3):177-81.
21. Verrotti A, Coppola G, Parisi P, Mohn A, Chiarelli F. Bone and calcium metabolism and antiepileptic drugs. Clin Neurol Neurosurg 2010;112(1):1-10.
22. Musso CG, Vilas M, Aparicio C, Bevione P, Reynaldi J, Rojas J, *et al*. Oxcarbazepine induced hyponatremia: A potenial explanation of its physiopathology. Rev Electron Biomed Electron J Biomed 2009;1:32-5.
23. Abd El-Wahab G, Hamdi H, Eleyan M. Teratogenic effects of the anti-epileptic drug (Levetiracetam) on albino rat fetuses during pregnancy and lactation. RJPBCS 2015;6(1):1456-74.
24. Aycicek A, Iscan A. The effects of carbamazepine, valproic acid and phenobarbital on the oxidative and antioxidative balance in epileptic children. Eur Neurol 2007;57(2):65-9.
25. Michoulas A, Tong V, Teng XW, Chang TK, Abbott FS, Farrell K. Oxidative stress in children receiving valproic acid. J Pediatr 2006;149(5):692-6.
26. Agarwal NB, Agarwal NK, Mediratta PK, Sharma KK. Effect of lamotrigine, oxcarbazepine and topiramate on cognitive functions and oxidative stress in PTZ-kindled mice. Seizure 2011;20(3):257-62.
27. Deepa D, Jayakumari N, Thomas SV. Oxidative stress is increased in women with epilepsy: Is it a potential mechanism of anti-epileptic drug-induced teratogenesis? Ann Indian Acad Neurol 2012;15(4):281-6.